

**RESEARCH ARTICLE**

**EFFECT OF ETHANOLIC AND AQUEOUS EXTRACT  
OF LEAF OF *CENTELLA ASIATICA* LINN ON IMMUNOMODULATORY PROPERTIES**

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**INTRODUCTION**

In traditional medicine (*Ayurveda*), there are several plants or constituents which are useful in preventive medical care, aimed at improving the quality of life and longevity. These constituents are known as *Dravya Rasayanas*. *s*. The *Rasayanas* are also claimed to increase the resistance of body to infection and other external factors tending to perturb the homeostasis of the human system, promote revival of physiological functions after debilitating diseases and to augment intellect. It is, therefore apparent that there is a remarkable similarity between the Ayurvedic concept of *Rasayanas* and the modern concept of adaptogens.

A list of Ayurvedic medicinal plants showing immunomodulatory activity has been provided which includes agents like *Withania somnifera*, *Allium sativum*, *Azadirachta indica*, *Piper longum*, *Asparagus racemosus*, *Glycyrrhiza glabra*, *Aloe vera*, *Gmelina arborea*, *Tinospora cordifolia* etc.<sup>2</sup> to possess immunopotential or adaptogenic action but experimental work on immunomodulation has not been dealt with. Immunomodulators may act at various steps / levels of the overall immune network. Therefore, the aim of the present study was to evaluate the effect of on immunomodulation in experimental models as also in toxicological profile.

***Centella asiatica* commonly known as *Thankuni* in Bengal. The medicinal value of the plant is mentioned in historic Vedic Literature.**

It is of Apiaceae family and found in East south east and to some extent south west Asia. The traditional uses of the medicinal plants are in treatment of weight loss, enteric problems, neurodegenerative diseases and memory loss, wound healing. The chemical composition has roughly been estimated to include acids like pectic acid, centotic acid, centellic acid, alkaloids viz. Hydrocotyline, vellarine, sterols like betasterol, gamma sitosterol, glycoside like asiaticoside, resinous substances and fat.

The present study was conducted to elucidate the immunomodulatory role of the plant as growing body of evidence indicates that the underlying mechanism of the medicinal effects against many diseases are the immunomodulatory activities and the various immune responses. No scientific data has been presented without minor exceptions relating to this plant which is of widespread medicinal use in India and other Asian countries.

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## MATERIALS AND METHODS

**Plant Extract:** The plant was collected from the local market in Kolkata and the plant identified by Botanical survey of India. A voucher specimen of the plant material is being kept in the Biochemistry Department of the concerned author.

**Preparation of Ethanolic Extract:** The ethanolic extract was prepared using 95% ethanol and 0.90Kg/lit of solvent. The ethanol extraction was made at room temperature of 37°C in rotary evaporator under sealed condition. The extracts with suitable adsorbents were stored at 4°C until further experiments.

**Preparation of Aqueous Extract:** The aqueous extract was prepared using 5% ethanol and 0.90Kg/lit of solvent. The aqueous extraction was made at room temperature of 37°C in rotary evaporator under sealed condition. The extracts with suitable adsorbents were stored at 4°C until further experiments.

**Preparation of the test drug:** The ethanolic extract was dissolved in aqueous medium according to the concentration as mentioned below and this is subsequently referred to as the "Test Drug".

The Test Drug prepared from ethanolic extract is referred to as Test Drug A and Test Drug prepared from aqueous extract is referred to as Test Drug B.

**Animals:** Adult Swiss albino male mice (25-30 g) and adult male Wistar rats (150-200 g) were used in these study. They were supplied pellet diet (Lipton, India) and water *ad libitum* and housed in ambient room temperature in our air-conditioned and well ventilated departmental animal house.

**Test Drug:** were dissolved in double distilled water for conducting the experiments. Two doses, 0.5 g/kg and 1.0 g/kg, orally, once per day were chosen through out the experiment as the selected dosages.

**Determination of Lethal Dose:** According to the screening procedure of Turner (1965),<sup>3</sup> the test drugs were given orally to pairs of rats in ascending doses up to 5 g/kg. The rats were observed continuously for 4 h. The rate of mortality up to 72 h was recorded.

**Body Weight:** The rats were treated with the test drugs at the above mentioned doses for 30 consecutive days, whereas, control rats received only distilled water (5 ml/kg). The body weight of the each animal was taken occasionally and presented in Table 1.

**Hematological Profile:** The rats were given test drugs once per day at the above mentioned doses for 10 consecutive days. Control rats received only distilled water (5 ml/kg). At the end of the experiment, the animals were sacrificed and blood was collected. Hemoglobin (Hb) concentration, total counts of red blood cells (RBC), white blood cells (WBC) and packed cell volume (PCV) were estimated.<sup>4</sup>

**Peritoneal Macrophages Count:** The albino mice were pretreated with test drugs orally for 10 days. The control group received only distilled water (5 ml/kg). At the end of the pretreatment, the mice were sacrificed and 8 ml of phosphate buffer saline (pH 7.2) was injected in the peritoneum cavity. The peritoneum fluid was collected, incubated at 37°C for 1h, centrifuged and phagocytes were counted by the uptake of 1% neutral red solution.<sup>5</sup>

**Carbon Clearance Assay:** The mice were pretreated for 10 days with test drugs, while the control group received only distilled water. After the pretreatment, 0.1 ml of colloidal carbon was injected in the tail vein of all the mice. At different intervals, a drop of blood was collected from retro-orbital plexus and 10 µl of this sample was lysed in 2 ml of 1% acetic acid. The blood samples collected were spectrophotometrically measured at 650 nm.<sup>6</sup>

**Immunopotentiating Effect:** The rats were divided into four groups for each drug. Group A and B received distilled water, while Group C and D received test drug for nine days. At the day 9, all animals were immunized with 2% of sheep red blood cells (SRBC) in phosphate buffer (7.2). The treatment of test drug were continuing as before up to another five days. Two days later, Group B, C and D were administered Cyclosporine A (50 mg/kg, p.o). Sixth day after SRBC injection, all animals were sacrificed and blood was collected. Serial two fold dilution of serum samples were made in normal saline containing bovine serum albumin (0.1 %). Then, 25 µl of this diluted serum was mixed with 25 µl of 1% SRBC in phosphate buffer (pH 7.2) in microtitration plates. The mixture was allowed to stand at 37°C for 1 h. The value of highest serum dilution carrying visible haemagglutination was taken as the antibody titre expressed in terms of number of wells.<sup>5</sup>

**TABLES**

**Table - 1:** Effect of Ethanolic and aqueous extract of *Centella asiatica* Linn on body weight gaining in rats

Body weight increase ( g )						
	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	3.2±0.004	8.4±0.008	13.0±0.005	16.5±0.001	20.7±0.002	26.9±0.007
0.5 g/kg of Test Drug A	4.6±0.002*	12.4±0.004*	18.6±0.004*	22.4±0.005*	28.6±0.002*	36.5±0.006*
1.0 g/kg of Test Drug A	5.3±0.005*	14.3±0.003*	20.9±0.001*	27.3±0.002*	35.3±0.001*	42.4±0.005*
0.5 g/kg of Test Drug B	4.3±0.003*	11.6±0.001*	16.5±0.004*	20.3±0.006*	26.0±0.003*	34.3±0.004*
1.0 g/kg of Test Drug B	5.5±0.002*	13.8±0.005*	19.2±0.002*	25.9±0.004*	31.4±0.002*	39.2±0.007*

N=6 in each group; values are mean±SEM; \*P<0.001 when compared to the control (Student's t-test)

**Table - 2:** Effect of Ethanolic and aqueous extract of *Centella asiatica* and on hematological parameters in rats

	Control	Test Drug A		Test Drug B	
		0.5 g/kg	1.0 g/kg	0.5 g/kg	1.0 g/kg
Hb%	14.2±0.62	14.4±0.80	14.6±0.46	14.3±0.50	14.5±0.28
RBC x 10 <sup>6</sup>	8.5±0.72	8.62±0.68	8.80±0.50	8.58±0.44	8.70±0.82
WBC x 10 <sup>3</sup>	13.8±8.50	14.2±7.62	14.5±6.72	14.5±5.88	14.6±6.02
PCV	35.2±3.28	35.6±6.72	35.9±5.32	35.2±3.60	35.7±7.82

N=6; values are Mean±SEM; all values show statistical non significance

**Table - 3:** Effect of ethanolic and aqueous extract of *Centella asiatica* On carbon clearance assay in mice

Optical absorbance at different duration (min)						
	0	5	15	30	45	60
Control	0.069	0.132±0.002	0.298±0.008	0.265±0.001	0.190±0.004	0.135±0.005
Test Drug A 0.5 g/kg	± 0.068	0.105±0.001*	0.208±0.009*	0.182±0.004*	0.160±0.008*	0.112±0.002*
Test Drug A 1.0 g/kg		0.95±0.003*	0.187±0.005*	0.165±0.002*	0.140±0.005*	0.096±0.003*
Test Drug B 0.5 g/kg		0.108±0.002*	0.215±0.008*	0.196±0.005*	0.175±0.006*	0.119±0.004*
Test Drug B 1.0 g/kg		0.098±0.008*	0.190±0.001*	0.171±0.007*	0.138±0.003*	0.102±0.006*

N=8 in each group; values are mean±SEM; \*P<0.001 when compared to the control (Student's t-test)

**Table - 4:** Effect of ethanolic and aqueous extract of *Centella asiatica* on peritoneal macrophages in mice

Total peritoneal macrophages count (cell/mm <sup>3</sup> )		
	Day 0	Day 10
0.5 g/kg of Test Drug A	1831±25.60	3829±30.25*
1.0 g/kg of Test Drug A		4275±68.50*
0.5 g/kg of Test Drug B		3760±26.70*
1.0 g/kg of Test Drug B		4075±52.68*

N=6 in each group; values are mean±SEM;

\*P<0.001 when compared to Day 0 (Student's t-test)

**Table - 5:** Effect of ethanolic and aqueous extract of *Centella asiatica* and on aemagglutination antibody titre in Cyclosporine A (CLY) induced immuno-deficient rats

Different dilution (SRBC : diluted serum)											
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
SRBC	+	+	+	+	+	+	+	+	+	-	-
SRBC+CYL	+	+	+	+	-	-	-	-	-	-	-
SRBC+CYL+ Test Drug A 0.5g/kg	+	+	+	+	+	+	+	-	-	-	-
SRBC+CYL+ Test Drug A 1.0g/kg	+	+	+	+	+	+	+	+	-	-	-
SRBC+CYL+ Test Drug B 0.5g/kg	+	+	+	+	+	+	+	-	-	-	-
SRBC+CYL+ Test Drug B 1.0g/kg	+	+	+	+	+	+	+	+	-	-	-

N=6 in each case

## RESULTS AND DISCUSSION

Herbal Preparations are becoming increasingly popular for a variety of diseases and infective conditions in recent times. A factor which influences the recovery from such an infective process is the host defiance mechanism. Immunomodulators have potentials to modulate an immune response. Immunomodulatory agents of plant or natural origin enhance the immune responsiveness of an organism against a pathogen by non-specifically activating the immune system. and prepared on the basis of *Rasayana* Ayurvedic formulation by Kolkata. Rasayana is meant prevention of diseases as well as promotion of positive health.

In the present context, it has been observed that the tested drugs did not produce any signs of toxicity or mortality. However, rats fed with beyond 5 g/kg b.w., experienced no mortality and so LD<sub>50</sub> of could not determined. Therefore, have no acute lethal effect.

improved body weight in animals but, did not alter peripheral blood cells morphology or count (Table 1-2). By enhancing body weight supported the ancient literature, *Charak Samhita*, which claiming the anabolic action of of many medicinal herbs if indigenous origin.

Microorganisms or their experimental equivalent of carbon particles, are readily engulfed by circulating and tissue fixed phagocytes. In vivo it is impossible to predict which of the phagocytic compartments was involved in the clearance of different particles.<sup>5</sup> In the present program, exhibited high macrophage activity as judged by carbon clearance test (Table 3). Macrophages are present throughout the connective tissue and in the lung, liver, spleen, lymph where they are strategically placed to filter off foreign material. Similarly, polymorphonuclear neutrophil is the dominant white cells in the bloodstream. In this context, and were further examined to find the ability of these drugs to increase the normal peritoneal macrophage count. The results indicated that and significantly enhanced the number of peritoneal macrophages and maximum number of cell was found to be on the 10 th day of drug administration (Table 4). These macrophages not only carry antigen and interact with lymphocytes but also non-specifically kill the invading organisms. Apart from this unlike neutrophils, macrophages are capable of protein synthesis, cell division and upon stimulation with agents like immunostimulants may divide rapidly from a cell line with a long life, perhaps months. This cooperation with lymphocytes induces immune response and reduces the chance of re-infection.

Cyclosporin A is a well known drug used clinically in immunosuppression during hyper-reaction. Cyclosporin A at higher doses are used in experimental researches for the searching of immunostimulant or immunomodulatory agents. Immunostimulant effect offers promise in enhancing antigen specific and non-specific immune response against infection. When an antigen solution is mixed in correct proportions with a potent antiserum, a precipitate is formed. Antibody, complement and polymorphs give protection against most extracellular organisms. In the present study, Test Drug A and Test Drug B, or the extracts of *Centella asiatica* Linn significantly protected Cyclosporine A induced humoral immunosuppression in rats (Table 5), which suggested the immunoprotective role of as well as the non-specific immunomodulatory property of them. However the mechanism of induced immunomodulation are not known and require further extended study.

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